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RESEARCH ARTICLE

IDENTIFICATION OF ANTIOXIDANTS IN *ALCHORNEA CORDIFOLIA* (SCHUMACH. & THONN.) MÜLL. ARG, A CANDIDATE PLANT FOR PHYTOREMEDIATION OF POLLUTED SOILS WITH MANGANESE.

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Abstract

The aim of this work was to establish antioxidants biochemical profile of *Alchornea cordifolia* vitro-plants, grown on substrate with different manganese concentrations, for the suitable use of regenerating in a phytoremediation program of soils polluted by this element. Conductivity and pH including essential parameters in assimilation of minerals by the plant were studied on substrate cultures. There was no significant difference in these two parameters on the four substrates tested, with an average of 4.7 and 188.63 µS/cm, respectively for pH and conductivity. Guaicol peroxidase (GOPX) activity was higher in leaves and stems, on media with high concentrations of Manganese (Mn) residues. In substrates containing low concentration of Mn, this activity was more important on roots. As for Glutathione (GSH), its activity was also greater on the leaves with a maximum value of 2.356 nmol/g on the substrates.

Introduction:-

Human activities, particularly mining, generate a large amount of metal-fortified waste. Their dispersion in soils is a danger for flora, fauna and especially for humans, the latter being the talei of the food chain (Sinha et al., 2013). Due to their persistence toxicity with increasing levels in the environment, metals represent one of the most important environmental pollution problems in the world (Delcarte et al., 2013). The rehabilitation of polluted soils with physico-chemical methods of treatment, are often expensive and generally appropriate for the decontamination of very localized areas, presenting an acute pollution and / or with strong land pressure. These depollution techniques affect the biological activity of soils and degrade their structure, leaving residues. Although technologies are currently available for water and soil treatment, strong interactions with the organo-mineral matrix makes it difficult to extract or inactivate soil pollutants from available technologies without altering their properties. The biological methods of soil decontamination, appears as an alternative method or complementary technique, less expensive and guaranteeing the characteristics of soils (Hooda, 2007). Phytoremediation is an alternative method for the remediation of polluted soils through the use of plants. Over the past decade, it has attracted growing interest. This new technology exploits the potential of plants and their associated microbiota to extract, stabilize or volatilize heavy metals present in soils. It is regularly mentioned in the scientific community as a promising solution. This process exploits the different physiological mechanisms of plants that allow them to grow in a contaminated environment.The implementation of a strategy for the management of soils polluted by manganese in the extraction sites and their environment, one of the phases of sanitation and rehabilitation by phytoremediation, concerns the

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identification then the characterization of appropriate plant material to meet the objectives of sustainable management. Preliminary work carried out on banks of Moulili River with, running through a manganese exploitation concession and heavily polluted by this element in southeastern Gabon has shown that *Alchornea cordifolia* (Schumach & Thonn.) Müll.Arg, a plant of the family Euphorbiaceous, has good potential for phytoremediation. This plant is able to accumulate manganese on leaves (unpublished result) and its powerful root system can stabilize the banks.It is therefore an ideal candidate to extract this metallic trace element (MTE) but also to stabilize the banks of this river. The present study aims to identify some phytochemical elements, produced by this plant in a manganese polluted environment.

Material and Methods:-

1.1 *Plant material and substrate*

The plant material consists of one-year-old *Alchornea cordifolia* young plants, obtained by *in vitro* culture, acclimatized and grown under polyethylene greenhouse condition at the campus of Masuku University of Science and Technology in the Higher National Institute of Agronomy and Biotechnology (geographical coordinates; 1°37'50,29 "S and 13°33'11, 38"E). The growth substrate for the *Alchornea* vitro-plants consists of an underground soil containing 0, 25, 50, 75 and 100 % of manganese residues.

1.2 *pH and conductivity*

The pH and conductivity were measured by the electrometric method, according to the ratio 1/5 (m: v) (Wilberforce, 2016). A quantity of 50 ml of demineralized water was added to 10 g of dried soil sample, the mixture was homogenized using a magnetic agitator (VARIOMAG®, Electronicrührer POLY 15) for 1 hour. Then the samples were incubated overnight. Finally pH and conductivity measurements were made with a multifunction pH meter (OAKTON, PC 700).

1.3 *Protein extracts preparation*

The leaves, stems and roots of *A. cordifolia*, from different culture substrates were washed with distilled water. 0.5 g of each plant sample, was crushed in a mortar at 4 ° C., weighed in a solution at pH 7, containing 0.5 ml potassium phosphate buffer (0.1 M), 0.1 mM EDTA, 0.1 mM ascorbate and 1% polyvidone 25. The homogenate was then centrifuged at 3000 rpm for 10 minutes. The supernatant obtained represented the crude extract on which the enzymatic activities were measured. The crude extract was stored at - 4 ° C.

1.4 *Quantitative analysis of Guaicol peroxidases (GOPX)*

The assay is performed with pyrogallol as substrate for Guaicol peroxidases. In presence of hydrogen peroxide, peroxidases oxidized to pyrogallol (phenol compound) to form a colored product called purpurogallin. Its appearance was observed on a spectrophotometer at 470 nm. The intensity was proportional to enzyme activity. 0.5 ml of protein extract was added to 2 ml of the reaction mixture consisting of 10^{-2} M pyrogallol-hydrogen peroxide. The blank was prepared under the same conditions by replacing the extract with 0.5 ml of 0.5 mL of 0.1 phosphate buffer and pH 7.0 in a tube. These tubes were rapidly stirred and incubated for 5 min in dark at 25 ° C. The enzymatic reaction was stopped by transfer of the tubes in a boiling water bath for 5 min. After cooling, optical density was measured at 470 nm with the UV-Vis spectrophotometer (Thermo Scientific, Genesy Bio). The activity of Guaicol peroxidase was expressed in µmol of pyrogallol/min/g of protein.

1.5 *Quantitative analysis of Reduced Glutathione (GSH)*

Reduced glutathione (GSH) was determined using the method according to Moron et al. (1979). Reduced glutathione reacted with 5,5'-dithiobis nitro benzoic acid (DTNB) giving a yellow colored product which absorbed at the wavelength of 412 nm. A homogenate was prepared with 0.5 g of plant sample in 5 ml of 5% trichloroacetic acid (TCA). The protein precipitate was centrifuged at 1500 rpm for 10 minutes. The supernatant was reduced to 1.0 ml with 0.1 M potassium phosphate buffer (pH 8.0). The standard GSH corresponding to the concentrations of 2 and 10 nmol was prepared. 2 ml of a solution of DTNB (0.6 mM in 0.1 M of potassium phosphate buffer, pH; 8) freshly prepared were added. The intensity of the yellow colour was measured in a UV-Vis spectrophotometer (Thermo Scientific, GENESY 10 Bio) at 412 nm after 10 minutes. The standard curve was made from GSH Standard (10 nmol / ml of 5% TCA). The values obtained were expressed in nmoles of GSH/g of sample.

1.6 *Statistical ANALYZES*

With three replicates for each analysis, the collected data were analyzed using the R (i386 3.3.3) software. They were then subjected to variance analysis at the 5% threshold. The Newman and Keuls test was used for averages comparison.

Results and discussion:-

2.1 *pH AND CONDUCTIVITY*

The results relating to chemical parameters of the substrates used for growth of *A. cordifolia* are shown in Table 1.

Tableau 1:-pH and electrical conductivity values of growth substrates

Manganese residues (%)	pH	Conductivity (µS/cm)
0	4.77 [a] ± 0.43	204.28 [a] ±96.63
25	5.26 [a] ±0.18	153.20 [a] ±41.38
50	5.08 [a] ±0.31	217.10 [a] ±73.17
75	4.92 [a] ±0.24	148.43 [a] ±63.70
100	5.77 [a] ±0.40	120.15 [a] ±46.81

Means with the same letter are not significantly different (α=0.05), with P-value $_{conductivity}$ = 0.0993 and P-value $_{pH}$ = 0.0601.

The pH values of substrates ranged from 4.77 ± 0.43 to 5.77 ± 0.40. The difference between this different in pH is less than one unit. They were all within the relative tolerance range of tropical plants between 4 to 7.5 (Pieri and Moreau 1987). Statistical analysis showed that there are no differences in the pH of substrates that contain different concentrations of manganese. The pH range of tested substrates, was also favorable to assimilation nutrients for acidophilic plants, although values close to neutrality are often a criterion for a good substrate culture. It should be noted that the optimum pH of *A. cordifolia* has not been determined yet.

The pH is a factor whose role is crucial for metal ions mobility, because it influences the number of negative charges that can be dissolved. The variation of the natural or anthropic pH seems to be the factor whose action on mobility metals is the most determining. Its decrease favors the mobility of metallic trace elements, in particular by dissolving metal salts or destroying the retention phase. Conversely, increasing the pH causes immobilization by formation of insoluble compounds or increased anion exchange capacity (Gleyzes et al., 2002). The pH variations therefore have complex and sometimes opposite consequences on the mobility of heavy metals, particularly in the presence of organic and inorganic ligands (Kushwaha et al., 2016). It is a fundamental element in controlled nutrition, because it determines assimilation conditions of mineral elements. Poor pH control is a regular factor in cyclamens accidents (pH <5.6). Manganese intoxication and other elements such as aluminum are often correlated with the presence of acidic pH in the presence unstable and fermentable materials.

The substrates conductivity varies from 120.15 ± 46.81 to 217.10 ± 73.17 µS/cm on the four media containing Mn. Its value is 204.28 ± 96.63 µS/cm on the control. There was also no significant difference in this parameter. The P-value was equal to 0.0993, therefore greater than the threshold 0.05, hence no significant difference in conductivity between averages. Manganese gradients don't influence conductivitry. Soils evaluated were sandy-clayey, acidic and low in organic matter (42.7%) with a bioavailable Mn concentration of 3406 mg/kg (Eba et al., 2007). Manganese migration depends on soil characteristics: in soils with a low cation exchange capacity (sandy acidic soils, for example), leaching is moderate; soils or horizons rich in organic matter would bind manganese weakly, whereas soils rich in clay would fix manganese rather strongly. Conductivity influences osmotic pressure and water absorption by cyclamen and may be responsible for physiological dryness. Consistent conductivity, guarantees the good vigor and vegetative balance of the plant. High conductivity makes it possible to maintain a compact plant habit. Figure 1 shows the variation of the two parameters as a function of the substrate (concentration of manganese residues).

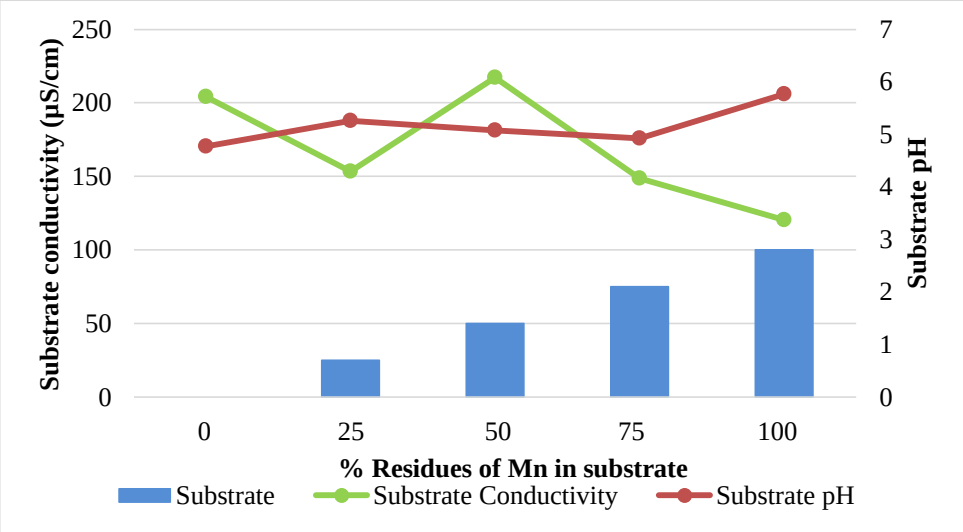

Figure 1 :- Effect of different concentrations of Mn residues on electrical conductivity and pH of substrates

A negative correlation between conductivity and pH is observed as a function of the concentration of Mn present in the substrate. An increase in pH is accompanied by a decrease in the substrate conductivity and vice versa. For phytoremediation of polluted soils, the removal of manganese by the root hairs depends on the characteristics of the soil, among which the pH or the cation exchange capacity, which is determined by the conductivity. A low soil pH increases the phytoavailability of manganese, because the H^+ ion, which has a greater affinity with the negative charges of colloids comes into competition with the metal ions opposite these sites, thus inducing a release of manganese into the soil water solution.

2.2 *GUAICOL PEROXIDASES (GOPX).*

Guaicol peroxidases activity in *A. cordifolia* was greater on substrates, having a Mn concentration less than or equal to 25 %. The optimum activity was recorded on the leaves with 0.168 µmol pyrogallol/min/g of protein on the substrate containing 75% Mn and the minimum in the roots is 0.0399 µmol pyrogallol/min/g of protein. This activity decreases throughout the plant on all substrates so the manganese concentration is greater than 25% and less than 100%. The activity of Guaicol peroxidase became greater in the roots on the substrate containing 100% Mn (0.0750 µmol of pyrogallol/min/g of protein) while it was lower in the stems and leaves, being 0.0414 µmol of pyrogallol/min/g of protein (Figure 2).

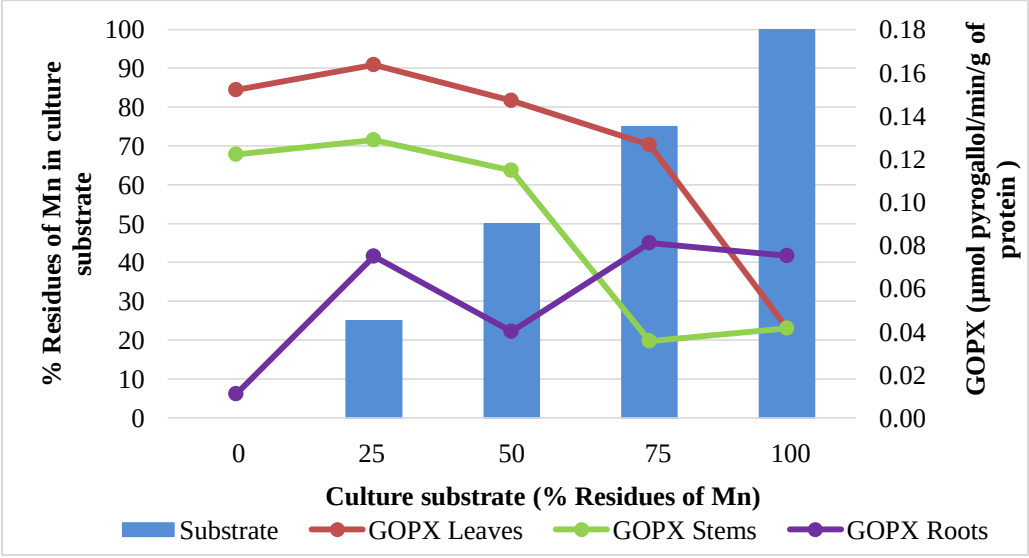

Figure 2 :-Guaicol peroxidases activity in leaves, stems and roots of *A. cordifolia* grown in different substrates

Guaicol peroxidase is an enzyme that uses hydrogen peroxide to oxidize its substrate. It participates in the defense of the cells against very toxic peroxides (Mavoungou et al., 2015). The activity variation of GOPX illustrates its redox potential on Mn. The presence of GOPX activity indicates that its production is an integral part of manganese detoxification process in *A. cordifolia*. Its decrease in leaf and stem activity indicatess migration and the preferential concentration of manganese in the leaves, in case of exposure of this plant to high doses of manganese (Eba et al., 2007).

2.3 *GLUTATHIONE (GSH)*

GHS activity in the plant was lower at the root level. It decreases as the concentration of Mn increases in the substrate. At the level of stems, this activity evolves irregular with a minimum on plants of the control substrate and an optimum on substrate containing high concentrations of manganese 75%. Glutathione is more active in the leaves. It reaches 2.356 nmol GSH/g on the substrate containing 100% Mn (Figure 3).

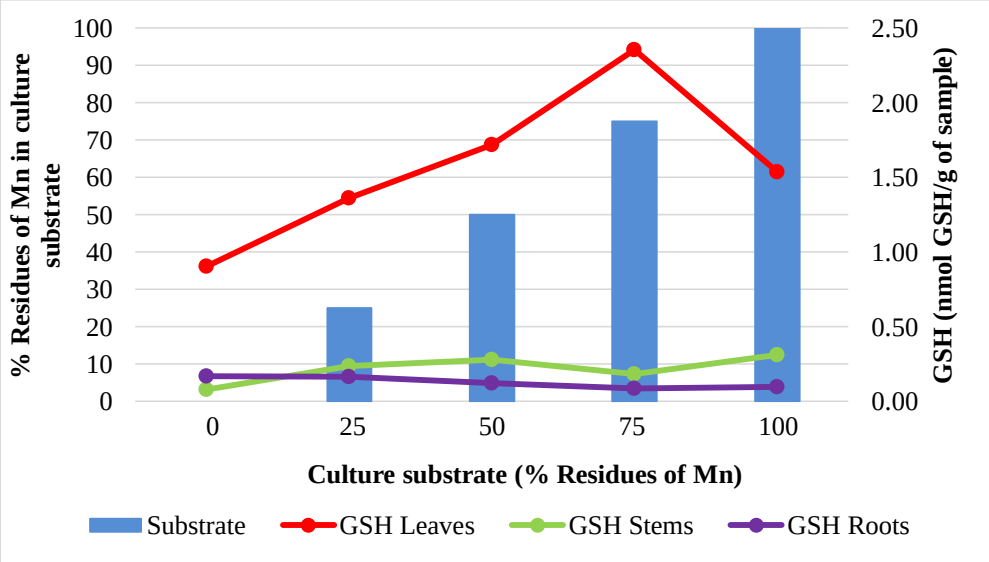

Figure 3:- GSH at each organ of the plant

GSH is a non-enzymatic antioxidant that monitors the hydrogen peroxide (H_2O_2) content in the plant. The degradation of H_2O_2 allows glutathione to control the cellular redox status of the plant, which contributes to the detoxification and balance of metals (Török et al., 2015). GSH is the substrate responsible for phytochelatines biosynthesis. PCs production and metal accumulation induces the production of a high level of glutathione. These phytochelatins mediate the vacuolar sequestration of metal-PC complexes. Mn being preferentially stored at the leaf level, this would justify the fact that GSH activity is higher in the leaves. In plants, phytochelatine-bound metals enter the cell through specialized transporters (Lui et al., 2015) such as adenosine triphosphate (ATP) bound to transport proteins. The metals are then stored in subcellular compartments such as the vacuole. Sequestration of metals in the vacuole illustrates part of the tolerance mechanism of some metal hyperaccumulators (Krämer, 2005). GSH therefore functions as a cellular sensor to ensure the maintenance of NADPH levels while ensuring the metabolism of different redox compounds.

Conclusion:-

Alchornea cordifolia seems to have the characteristics necessary for phytoremediation soils polluted with manganese residues. It is very efficient in environments contaminated by this type of pollutant and in extreme conditions. In addition, its high biomass production of 2 to 3 tonnes per hectare per year would allow the extraction / accumulation of target MTE that it would preferentially assimilate. Antioxidant activity of GOPX remains high during exposure to Mn. The presence of glutathione in different parts of the plant, more on the leaves where they are stored suggests that this antioxidant, which is a co-substrate of enzymes, would intervene in the degradation pathways of reactive oxygen species, assimilation and storage of Mn. Since the symptoms of manganese toxicity are not very visible during plant development cycle on the contaminated substrates, it is essential that further work be

done to identify the storage sites of this MTE in the plant, before considering the introduction of *Alchornea cordifolia* in a phytoremediation plan.

References:-

1. Delcarte, J., Gossiaux L., and Aricia E. (2013): Valorisation énergétique du bois produit à des fins de phytoremédiation. Renaturation des berges de cours d'eau et phytoremédiation. Presses agronomiques de Gembloux, pp. 107-19.
2. Eba, F., Ondo, J.A., Emané-Mba S., Ollui-M-Boulou M., and Omva-Zué, J. (2007): Taux de manganese accumule dans quelques plantes vivrieres cultivees dans la region manganesifere de Moanda (Gabon). Journal-Societe Ouest Africaine de Chimie, 23: 69.
3. Gleyzes, C., Tellier S. and Astruc M. (2002): Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. TrAC Trends in Analytical Chemistry, 21 (6): 451-467.
4. Hooda, V. (2007): Phytoremediation of toxic metals from soil and waste water. J. Environ Biol., 28:367-376.
5. Krämer, U. (2005): Phytoremediation: novel approaches to cleaning up polluted soils. Current Opinion in Biotechnology, 16 (2):133-141.
6. Kushwaha, A., Rani, R., Kumar, S. and Gautam, A. (2016): Heavy metal detoxification and tolerance mechanisms in plants: Implications for phytoremediation. Envion. Rev., 51:39-51.
7. Liu, Z., He, X., Chen, W. and Zhao, M. (2013): Ecotoxicological responses of three ornamental herb species to cadmium. Environmental Toxicology and Chemistry, 32(8): 1746-1751.
8. Mavoungou, A.Y., Lepengue A.N., and M'BATCHI B. (2015) : Antioxydants et phytochelatine dans la tolérance et l'accumulation du manganèse chez Hibiscus sabdariffa Linn. European Scientific Journal, 11(21): 1857- 7431.
9. Moron M.S., Depierre J.N., Mannervick V.C. (1979): Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Bioche Biophys Acta, 582: 67-68.
10. Pieri, C., and Moreau R. (1987): Fertilité des sols et fertilisation des cultures tropicales: l'expérience. Cirad-Orstom, 28: 367-376.
11. Sinha, S., Mishra, R.K., Sinam, G., Mallick, S., and Gupta, A.K. (2013): Comparative evaluation of metal phytoremediation potential of trees, grasses, and flowering plants from tannery-wastewater-contaminated soil in relation with physicochemical properties. Soil and Sediment Contamination, 22(8): 958-983.
12. Török, A., Gulyás, Z., Szalai G., and Kocsy, G. (2015): "Phytoremediation Capacity of Aquatic Plants Is Associated with the Degree of Phytochelatin Polymerization." *Journal of hazardous materials* 299: 371–78.
13. Wilberforce J.O.O. (2016). Accumulation of Toxic Metals in Soils of Different Sections of Mechanic Village Abakaliki, Nigeria and Their Health Implications. American Chemical Science Journal 11(1): 1-8